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(54) Title: METHODS FOR IMPROVING THE FUNCTION OF HETEROLOGOUS G PROTEIN–COUPLED RECEPTORS

(57) Abstract

This invention relates to mutant G protein–coupled receptors with improved G–protein coupling and receptor response, yeast cells expressing such receptors, vectors useful for making such cells, and methods of making and using same.

METHODS FOR IMPROVING THE FUNCTION OF HETERLOGOUS G PROTEIN-COUPLED RECEPTORS

STATEMENT OF RELATED APPLICATIONS

This application hereby claims the benefit of United States provisional application Serial No. 60/098,704 filed September 1, 1998. The entire disclosure of this provisional application is relied upon and incorporated by reference herein.

FIELD OF THE INVENTION

This invention relates to mutant G protein-coupled receptors with improved G-protein coupling and receptor response, host cells expressing such receptors, vectors useful for making such cells, and methods of making and using same.

BACKGROUND OF THE INVENTION

The actions of many extracellular signals, such as neurotransmitters, hormones, odorants, and light, are mediated by a triad of proteins which has been identified in organisms from yeast to mammals. This triad consists of a receptor, coupled to a trimeric guanine nucleotide-binding regulatory protein (G protein), which in turn is coupled to a cellular effector. These receptors have seven transmembrane domains and are named for their association with the G protein as "G protein-coupled receptors" ("GPCRs").

The regulatory G proteins are comprised of three subunits: a guanylnucleotide binding α subunit; a β subunit; and a γ subunit. *B.R. Conklin and H.R. Bourne (1993)*. G proteins cycle between two forms, depending on whether GDP or GTP is bound to the α subunit. When GDP is bound, the G protein exists as a heterotrimer, the G$\alpha\beta\gamma$ complex. When GTP is bound, the α subunit dissociates, leaving a G$\beta\gamma$ complex. Importantly, when a G$\alpha\beta\gamma$ complex operatively associates with an activated G protein coupled receptor in a cell membrane, the rate of exchange of GTP for bound GDP is increased and, hence, the rate of disassociation of the bound Gα subunit from the G$\beta\gamma$ complex increases. The free Gα subunit and G$\beta\gamma$ complex are capable of transmitting a signal to downstream elements of a variety of signal transduction pathways. Examples of these downstream cellular effector proteins include, among others, adenylate cyclases, ion channels, and phospholipases. This

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fundamental scheme of events forms the basis for a multiplicity of different cell signaling phenomena. *H.G. Dohlman et al. (1991)*.

Because of their ubiquitous nature in important biochemical pathways, the G protein-coupled receptors represent important targets for new therapeutic drugs. In turn, the discovery of such drugs will necessarily require screening assays of high specificity and throughput termed high-throughput screening (HTS) assays. Screening assays utilizing microorganisms, such as yeast strains, genetically modified to accommodate functional expression of the G protein-coupled receptors offer significant advantages in research involving ligand binding to numerous receptors implicated in various disease states.

However, microorganisms transformed with wild-type receptors may perform poorly in growth assays, exhibiting, for example, the inability to interact with the heterotrimeric G protein, inappropriate localization and/or desensitization. Many GPCRs are phosphorylated in response to chronic and persistent agonist stimulation which often leads to desensitization followed by sequestration or internalization of the receptors. Desensitization of GPCRs causes uncoupling from interaction with heterotrimeric G proteins. This process is mediated by a variety of regulatory receptor protein kinases, including G protein-coupled receptor kinases (GRK), protein kinase A (PKA), protein kinase C (PKC), and casein kinases (CK). Internalization involves removal of GPCRs from the plasma membrane via receptor-mediated endocytosis. Internalized receptors may be recycled back to the cell surface, or delivered to a lysosomal/vacuolar compartment for degradation. The ubiquitin-mediated degradative pathway is also involved in this process. The ultimate result of receptor phosphorylation and sequestration/internalization is often cell growth arrest, which significantly reduces the utility of the genetically modified microorganism in screening assays.

SUMMARY OF THE INVENTION

It is an object of this invention to provide modified G protein-coupled receptors that function well in high throughput screening assays, implemented in any eukaryotic cell, preferably yeast cells. Thus, a first aspect of the present invention is

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directed to nucleotide sequences encoding a G protein-coupled receptor which has been modified to improve the function of the GPCR by causing the receptor to couple more efficiently with the heterotrimeric G protein and/or to fail to interact with the cell desensitization and/or sequestration/internalization machinery, and/or to appropriately localize to the plasma membrane. In preferred embodiments, such modifications lead to improved agonist-stimulated growth-promoting ability. One specific modification of the nucleotide sequence encoding a G protein-coupled receptor encompassed by this invention is a mutation in any intracellular domain or membrane region proximal to internal domains. The mutation may be a deletion, including, for example, a point mutation.

This invention is also directed to chimeric GPCRs in which intracellular domains of heterologous GPCRs that provide favorable G protein coupling properties or domains not subject to the yeast cell desensitization and/or sequestration/internalization machinery are used to replace comparable domains in GPCRs of interest. This invention also relates to the modified nucleotide sequences encoding the chimeric GPCRs, to expression vectors comprising the modified nucleotide sequences, and to host cells transformed therewith.

An additional aspect of this invention is an improved method of assaying compounds to determine effects of ligand binding to the mutant or chimeric GPCRs of this invention by measuring the effect of the test compound on cell growth. The mutant GPCRs prevent or reduce the rate of cell growth arrest due to chronic and persistent agonist stimulation, thereby decreasing the number of false negatives that occur with prior art screening methods and/or increasing the sensitivity of the bioassay.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1A depicts the results of liquid culture assays on yeast cells containing the rat M3 muscarinic acetylcholine receptor (MAR) using MAR agonist carbachol (CCh). Yeast cells containing a M3 MAR with a deletion in the third intracellular loop (IC3Δ) produced an agonist-dependent growth response, while the wild type MAR did not, indicating that the M3 MAR IC3Δ is functional GPCR.

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FIGURE 1B depicts the results of liquid culture fluorescence induction assays on yeast cells containing the rat M3 MAR IC3Δ and the FUS2-GFP reporter plasmid using the MAR agonist carbachol (CCh). A dose-dependent increase in the expression of the green fluorescent protein is observed in response to CCh activation of the M3 MAR IC3Δ expressed in yeast.

FIGURE 2 depicts the results of liquid culture assays on yeast cells containing the *Drosophila* muscarinic acetylcholine receptor using the MAR agonist carbachol (CCh). Yeast cells containing a mutated *Drosophila* MAR containing the M3 MAR IC3Δ produced an agonist-dependent growth response while the wild type *Drosophila* MAR lacked an agonist-dependent yeast cell growth response.

FIGURE 3 depicts the results of an agar-based plate bioassay. FIGURE 3A shows a robust growth response of yeast cells containing the IC3Δ cholecystokinin CCKB receptor. FIGURE 3B shows only limited growth by yeast cells containing the wild type CCKB receptor, indicating that the deletion of a portion of the third intracellular loop of the CCKB receptor improves its function in yeast.

FIGURE 4 depicts yeast cells transformed with rSSTR3 and with rSSTR3ΔIC3. FIGURE 4A demonstrates that yeast cells containing p426GPD-rSSTR3 show a weak response to somatostatin (S-14). FIGURE 4B demonstrates a much stronger response by yeast cells containing p426GPD-rSSTR3ΔIC3 assayed under similar conditions.

FIGURE 5 depicts the results of liquid culture assays on yeast cells containing wild type IC3Δ human alpha2A adrenergic receptor using the alpha adrenergic receptor full agonist UK14304. Yeast cells containing the wild type and IC3Δ human alpha 2A adrenergic receptor produced a dose-dependent growth response, indicating that the IC3 deletion is functional.

FIGURE 6 depicts the results of liquid culture assays on yeast cells containing wildtype and carboxy terminally truncated rat NT1-neurotensin receptors using the neurotensin receptor agonist AcNT8-13. Truncation of the rat NT1-neurotensin receptor produces an agonist-dependent growth response that is more sensitive than that observed with the wildtype receptor.

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FIGURE 7 depicts the results of liquid culture assays on yeast cells containing the *C. elegans* serotonin receptor using serotonin (5HT) to stimulate yeast cells growth. Yeast cells containing a mutated *C. elegans* serotonin receptor containing the M3 MAR IC3Δ produced a 5HT-dependent growth response. The growth response was blocked by addition of the serotonin receptor antagonists lisuride and mianserin.

DETAILED DESCRIPTION OF THE INVENTION

Modified G Protein-Coupled Receptors

Nucleotide sequences encoding G protein-coupled receptors may be modified to improve the function of the GPCR by causing the receptor to couple more efficiently with the heterotrimeric G protein and/or to fail to interact with the cell desensitization and/or sequestration/internalization machinery. Such modifications lead to improved agonist-stimulated yeast cell growth-promoting ability. The improvement of GPCR-G protein coupling and/or elimination of receptor phosphorylation and/or sequestration/internalization in the host cell provides a means to improve the function of wildtype heterologous GPCRs that fail to stimulate a useful yeast cell growth response. Thus, GPCRs that fail to function in their wild type form may be made to work by the methods of this invention.

The improvement of GPCR-G protein coupling and or elimination of receptor phosporylation and/or sequestration/internalization in the host cell may be assessed by using routine techniques, such as those described in the Examples set fort below or known to those of skill in the art. For example, improvement of the function of a mutated GPCR over wild type may be quantified as an increase in the signal-to-noise ratio and/or in the sensitivity of the liquid bioassay. The signal-to-noise ratio is determined by comparing the agonist-induced growth rate to the growth rate observed in the absence of agonist. A statistically-significant increase in the signal-to-noise ratio resulting from agonist-stimulation of a mutated GPCR over similar values obtained from cells containing a wild type receptor indicates that the function of the mutated GPCR has been improved.

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The sensitivity of the liquid bioassay is defined as the agonist concentration necessary to produce a half-maximal growth rate (ED50 or EC50). The sensitivity of the bioassay is increased if a mutated GPCR produces a half-maximal growth rate at an agonist concentration that is less than that required by the wild type GPCR.

Similarly, the more qualitative agar based bioassay will reflect increases in signal-to-noise ratio and/or sensitivity due to agonist stimulation of mutated GPCRs. In the agar based bioassay, signal-to-noise ratio increases are determined by comparing the extent of growth within the agonist induced growth zone resulting from stimulation of mutated and wild type receptor. The sensitivity of the bioassay is proportional to the radius of the growth zone. Since applied compounds diffuse radially from the site of application to the agar, agonist concentration varies with the square of the radius of the growth zone. Thus, a larger zone of growth in response to agonist activation of mutated GPCRs reflects an increase in sensitivity.

Any G protein-coupled receptor may be employed in practicing this invention. Examples of such receptors include, but are not limited to, adenosine receptors, somatostatin receptors, dopamine receptors, cholecystokinin receptors, muscarinic cholinergic receptors, α-adrenergic receptors, β-adrenergic receptors, opiate receptors, cannabinoid receptors, growth hormone releasing factor, glucagon, serotonin receptors, vasopressin receptors, melanocortin receptors, and neurotensin receptors. In certain preferred embodiments, the receptor is a muscarinic acetylcholine receptor and more preferably, the muscarinic acetylcholine receptor is of the M3 subtype.

Similarly, any suitable host cell may be transformed with the nucleotide sequences encoding the modified G protein-coupled receptors of this invention. Examples of suitable host cells are yeast cells, mammalian cells, insect cells, and bacterial cells. Preferably, the host cells are yeast cells.

One generalizable method for improving the function of a GPCR expressed in a host cell is by modification or elimination of intracellular domains of the GPCR, such as the third intracellular loop (IC3) sequences of the G protein-coupled receptor. Because the desensitization and internalization machinery acts upon the intracellular domains of the GPCR, elimination of the intracellular domains of the GPCRs produces a more stable receptor expression. This has been demonstrated in

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experiments conducted in mammalian cells. Muscarinic acetylcholine receptors, including the M3 subtype, lacking a domain of their third intracellular loop thought to be involved in receptor internalization, are maintained in the plasma membrane to a greater extent than their wild type counterparts. *Moro et al. (1993).*

Representative embodiments of the invention are described in more detail in the following examples.

Example 1.   Functional Expression of A Mutated Rat M3 Muscarinic Acetylcholine Receptor (MAR) in Yeast

The third intracellular loops of GPCRs are thought to interact with and participate in the activation of G proteins upon agonist binding. *J. Wess (1997).* Mutations in IC3 of the yeast mating pheromone receptors, Ste2 and Ste3 have profound effects on coupling the G proteins. *C. Boone et al. (1993)* and *C. Clark et al. (1994).* Importantly, deletion of portion of the IC3 of mammalian MARs, in particular the rat M3 MAR, is correlated with improved functional expression in mammalian cells with retention of full ability to couple to the heterotrimeric G protein, Gq (Gαβγ). The mutated M3 MAR retains all external loops. Transmembrane domains (TMDs) and internal domains other than the IC3 are unchanged. The IC3, found between 5th and 6th membrane spanning helices, was the only domain modified. The bulk of this domain, 96 amino acids in the center of the IC3 (Ala273-Lys469), were deleted, leaving only 22 amino acids proximal to both the 5th and 6th transmembrane helices. Thus, the third intracellular loop of the MAR containing the IC3 deletion (IC3Δ) is 44 amino acids in length, compared to 240 amino acids in the IC3 of wild type M3 MAR. The improvement in functional expression may due to elimination of domains known to interact with cellular desensitization mechanisms, allowing more functional MAR to be retained at the cell surface.

In order to test the possibility that this IC3Δ mutation would also improve functional expression in yeast, the DNA sequences encoding the wild type and IC3Δ rat M3 MARs were cloned into proximity to the glycerol-phosphate dehydrogenase promoter in the yeast expression plasmid, p426GPD, by standard methods. Rat M3

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MAR sequences were amplified by PCR using oligonucleotides containing 5' *Bgl*II
(AAAAGATCT AAA ATG TAC CCC TAC GAC GTC CCC) (SEQ ID NO: 1) and
3' *Xho*I (AAA CTCGAG CTA CAA GGC CTG CTC CGG CAC TCG C) (SEQ ID
NO: 2) sites. The resulting PCR product was digested with the appropriate restriction
endonucleases, purified and ligated into appropriate sites in p426GPD. To form the
rat M3 IC3Δ, three M3 MAR fragments were amplified by PCR. An amino-terminal
coding fragment was amplified using oligonucleotides containing 5' *Bgl*II
(AAAAGATCT AAA ATG TAC CCC TAC GAC GTC CCC) (SEQ ID NO: 1) and
3' *Age*I (ATAGTCATGATGGTG ACCGGT ATGTAAAAGGCAGCGATC) (SEQ
ID NO: 3) sites. A carboxy-terminal coding fragment was amplified using
oligonucleotides containing 5' *Pml*I (GCCTTCATCAT CACGTG
GACCCCCTACACC) (SEQ ID NO: 4) and 3' *Xho*I (AAA CTCGAG CTA CAA
GGC CTG CTC CGG CAC TCG C) (SEQ ID NO: 2) sites. An IC3 coding fragment
was amplified using oligonucleotides containing 5' *Age*I
(CGATCGCTGCCTTTTACTT ACCGGT CACCATCATGACTAT) (SEQ ID NO:
5) and 3' *Pml*I (GTTGTAGGGGGTC CACGTG ATGATGAAGGC) (SEQ ID NO:
6) sites using the M3 IC3Δ sequence. *J. Wess (1997)*. The resulting PCR products
were digested with the appropriate restriction endonucleases, purified and ligated into
appropriate sites in p426GPD. Plasmids were confirmed by restriction endonuclease
mapping and DNA sequencing. Using a conventional lithium acetate transformation
procedure, the resulting plasmids were introduced into yeast cells useful for
performing assays of GPCR agonist-stimulated growth, such as those described in
United States Patent 5,691,188, incorporated herein by reference, including,
specifically, the MPY578fc cells described in *Pausch et al. (1998)*.

Yeast cells containing the MARs were assayed in liquid culture using the
MAR agonist carbachol (CCh). The cells were cultured overnight in 2 ml SC-
glucose-ura medium. The cells were diluted 500 fold in SC-glucose-ura-his, pH 6.8
medium containing 5 mM 3-aminotriazole to decrease basal growth rate. Samples of
the cell suspension (180 μl) were dispensed to wells of sterile 96 well microtiter
dishes containing 20 μl of serially-diluted samples (10^{-1}-10^{-8} M) of the muscarinic
receptor agonists. The plates were incubated at 30°C for 18 hours with agitation (600

rpm). Growth was monitored by recording increases in OD_{620} using a microplate reader. Assays were conducted in duplicate and growth rate measurements obtained during the logarithmic phase of yeast cell growth. Optical density measurements were analyzed using GraphPad Prism and are presented as the mean ± SEM and were plotted vs. agonist concentration. As shown in Figure 1, the yeast cells containing the M3 MAR IC3Δ produced an agonist-dependent growth response, demonstrating that the M3 MAR IC3Δ is functional, while the wild type MAR is non-functional, as indicated by the lack of agonist-dependent yeast cell growth. The growth response of the M3 MAR IC3Δ containing cells was dose-dependent giving an EC_{50} for carbachol (CCh) equal to 3 μM. This value matches the K_D for CCh obtained in HEK cells (7.9 μM) and the EC_{50} for CCh induced IP_3 (inositol triphosphate) accumulation (4.0 μM), suggesting that the M3 MAR IC3Δ retains the expected pharmacological properties when expressed in yeast cell membranes. Further, the growth response is blocked by the MAR-specific antagonist, atropine (At).

Alternatively, the response to CCh by yeast cells containing the M3 MAR IC3Δ may be observed by measuring the agonist-induced increase in fluorescent emission from a green fluorescent protein reporter gene whose expression is stimulated by MAR agonists. Green fluorescent protein (GFP) is a protein from Aequorea that is intrinsically fluorescent when expressed in yeast cells. The fluorescence from GFP is detectable in live yeast calls, making it possible to measure the level of its expression without any deleterious treatment of the yeast cells. This feature is particularly advantageous in the reporter gene assays that do not require additional steps to permit its detection. An inducible reporter gene that is useful in detecting the agonist-activation of heterologous GPCRs expressed in yeast utilizes transcriptional promoters that are activated by the mating signal transduction pathway. One such promoter is the *FUS2* promoter. In the absence of agonist stimulation, little or no expression of the Fus2 protein or any other protein whose expression is directed by the *FUS2* promoter is detectable. After treatment with agonist, transcription from the *FUS2* promoter is induced up to 700 fold, leading to substantial increases in Fus2 expression or in the expression of any gene product whose expression is placed under control of the *FUS2* promoter. Thus, yeast cell fluorescence resulting from GFP

expression under the control of the *FUS2* promoter from a *FUS2*-GFP reporter gene is only observed after agonist activation of a heterologous GPCR.

In order to produce a GFP reporter gene, DNA sequences encoding the enhanced GFP (EGFP, Clonetech), *FUS2* promoter and *FUS2* transcriptional terminator sequences were amplified by PCR. The fragments were assembled into the centromere containing plasmid pRS414 so as to place EGFP expression under control of the pheromone responsive *FUS2* promoter in the centromere containing plasmid pRS414, producing plasmid pMP241. Using a conventional lithium acetate transformation procedure, the resulting plasmids were introduced into yeast cells of the kind described in United States Patent 5,691,188, that are useful for performing assays of GPCR agonist-stimulated growth of cells containing the M3 MAR IC3Δ. Specifically, the plasmids were introduced into the MPY578fc cells described in *Pausch et al. (1998)*.

Yeast cells containing the M3 MAR IC3Δ and the *FUS2*-EGFP reporter plasmid were assayed in liquid culture using the MAR agonist carbachol (CCh). The cells were cultured overnight in 2 ml SC-glucose-ura medium. The cells were washed and diluted 5 fold in SC-glucose-ura-his, pH 6.8 medium containing 5 mM 3-aminotriazole to decrease basal growth rate. Samples of the cell suspension (180 μl) were dispensed to wells of sterile 96 well microtiter dishes containing 20 μl of serially-diluted samples (10^{-1}-10^{-8} M) of CCh. The plates were incubated at 30°C for 6 hours with agitation (600 rpm). Stimulation of the *FUS2*-EGFP reporter gene expression was monitored by recording increases in emission at 530 nm after excitation with 480 nm light using a fluorescence microplate reader. Assays were conducted in duplicate and measurements obtained during the logarithmic phase of yeast cell growth. Fluorescence emission measurements were analyzed using GraphPad Prism and were presented as the mean ± SEM and were plotted vs. agonist concentration. As shown in Figure 1B, the yeast cells containing the M3 MAR IC3Δ produced a dose dependent increase in florescence emission, consistent with increased expression of the EGFP from the agonist inducible *FUS2*-GFP reporter gene construct. The EC50 for CCh stimulation of fluorescence emission is 4 μM, identical to values obtained in the growth assay.

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Thus, deletion of a portion of the IC3 of the rat M3 MAR has produced a

functional GPCR when expressed in yeast, suggesting that modification of internal

domains may be a generalizable method for improving the function of heterologous

GPCRs expressed in yeast.

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Example 2.   Functional Expression of a Mutated *D. melanogaster* Muscarinic Acetylcholine Receptor in Yeast

Agonist of the G protein-coupled insect muscarinic acetylcholine receptors

10   (MARs) possess substantial insecticidal and miticidal activity. *M.R. Dick et al.*

(1997). These observations suggest that development of a yeast-based high

throughput screen (HTS) for agonists active at insect MARs may be useful in

identifying lead compounds that might be developed into insecticides with novel

mode of action. Preliminary experiments indicate that the wild type *D. melanogaster*

15   MAR (DMAR), an insect G protein-coupled receptors (GPCRs), is non-functional in

yeast. Thus, an effort to develop a method for improving the function of the DMAR

in yeast was mounted, via replacement of the DMAR IC3 with the functional IC3

from the M3 MAR IC3Δ.

In insect cells, the DMAR interacts with the heterotrimeric Gq protein leading

20   to an increase in intracellular calcium in response to muscarinic agonists. One

potential explanation for the inactivity of the DMAR in yeast is an inability to

efficiently couple to the yeast heterotrimeric G protein. Thus, to devise a method to

improve the DMAR function in yeast, selected mutations in the GPCRs that serve to

improve functional expression and coupling to the heterotrimeric G protein were

25   examined.

In order to construct the IC3 replacement, PCR fragments encoding three

domains were prepared by standard means. Fragment 1 consisted of the amino

terminal coding portion of the *Drosophila* MAR up to an *Age*l site within the 5th

TMD, amplified by PCR using oligonucleotides (AAA AGATCT AAA ATG

30   TACGGAAACCAGACGAAC) (SEQ ID NO: 7) and (CCA GTA GAG GAA

GCACATGATGGTC AGGCCT AAG TAG AAG GCG GCC AGT GC) (SEQ ID

NO: 8). The second fragment of the DMAR was composed of carboxy terminal

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coding sequences starting with a *Pm*II site in the 6th TMD, amplified by PCR using oligonucleotides (TTCATCATCACGTGGACTCCGTACAACATC) (SEQ ID NO: 9) and (AAA CTCGAG TTATCTAATTGTAGACGCGGC) (SEQ ID NO: 10). The M3 MAR IC3Δ domain was amplified as an *Age*I-*Pm*II fragment with coding sequence in frame with fragments 1 and 2, described in Example 1. These fragments were assembled in plasmid p426GPD to place the mutated DMAR under control of the GPD promoter. The wild type DMAR was cloned into the expression vector, pMP3, described in United States Patent 5,691,188. Using a conventional lithium acetate transformation procedure, the resulting plasmids were introduced into yeast cells useful for performing assays of GPCR agonist-stimulated growth, such as those described in United States Patent 5,691,188, including, specifically, the MPY578fc cells described in *Pausch et al. (1998)*.

Yeast cells containing the DMAR and the plasmid containing the wild type DMAR were assayed in liquid culture using the MAR agonist carbachol (CCh). The cells were cultured overnight in 2 ml SC-glucose-ura medium. The cells were diluted 500 fold in SC-glucose-ura-his, pH 6.8, medium containing 5 mM 3-aminotriazole to decrease basal growth rate. Samples of the cell suspension (180 μl) were dispensed to wells of sterile 96 well microtiter dishes containing 20 μl of serially-diluted samples $(10^{-1}-10^{-8}$ M) of the muscarinic receptor agonists. The plates were incubated at 30°C for 18 hours with agitation (600 rpm). Growth was monitored by recording increases in OD620 using a microplate reader. Assays were conducted in duplicate and growth rate measurements obtained during the logarithmic phase of yeast cell growth. Optical density measurements were analyzed using GraphPad Prism and are presented as the mean ± SEM and were plotted vs. agonist concentration. As shown in Figure 2, the yeast cells containing the mutated DMAR, i.e., the M3 MAR IC3Δ, produced an agonist-dependent growth response, demonstrating that the DMAR-M3 MAR IC3Δ is functional. The wild type DMAR is non-functional, as indicated by the lack of agonist-dependent yeast cell growth.

Thus, replacing IC3 of the DMAR with the functional deleted IC3 from the rat M3 MAR produces a functional chimeric GPCR when expressed in yeast, indicating that this method of replacing internal domains may be a generalizable method for

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improving the functional expression of heterologous GPCRs in cell-based assays,
such as yeast assays.

Example 3.   Functional Expression of a Mutated Rat Cholecystokinin CCKB
               Receptor in Yeast

As shown in Examples 1 and 2, deletion of portion of the IC3 of mammalian
MARs, in particular the rat M3 MAR, is correlated with improved functional
expression in mammalian and yeast cells with retention of full ability to couple to the
heterotrimeric G protein. In order to test the possibility that this IC3Δ mutation would
also improve functional expression of other GPCRs in yeast, the DNA sequences
encoding the rat wild type and IC3Δ cholecystokinin CCKB receptor were amplified
by PCR and cloned into proximity to the glycerol-phosphate dehydrogenase promoter
in yeast expression plasmid, p426GPD, by standard methods. The wild type CCKBR
was amplified by PCR using oligonucleotides
(ACTTAGATCAAAAAATGGAGCGCTCAAGCTGAACCG) (SEQ ID NO: 11)
and (TCCCGTCGACTCAGCCAGGCCCCAGTGTGCTG) (SEQ ID NO: 12) . The
IC3Δ cholecystokinin CCKB receptor was prepared by fusing two overlapping
fragments. Fragment 1 contained amino terminal coding sequences including 22
amino acids proximal to the 5th TMD, amplified by PCR using oligonucleotides
(ACTTAGATCAAAAAATGGAGCGCTCAAGCTGAACCG) (SEQ ID NO: 11) and
(CGAGGGCCAGGGACTGGCCCCGGCCGGGCCCGGCTTTGGGTCTCG) (SEQ
ID NO: 13). Fragment 2 contained carboxy terminal coding sequences including 22
amino acids proximal to the 6th TMD, amplified by PCR using oligonucleotides
(TCCCGTCGACTCAGCCAGGCCCCAGTGTGCTG) (SEQ ID NO: 12) and
(CGAGACCCAAAGCCGGGCCCGGCCGGGGCCAGTCCCTGGCCCTCG) (SEQ
ID NO: 14). The two fragments were fused by amplification by PCR using oligos at
5' and 3' ends of the full length CCKB receptor. Using a conventional lithium acetate
transformation procedure, the resulting plasmids were introduced into yeast cells
useful for performing assays of GPCR agonist-stimulated growth, such as those
described in United States Patent 5,691,188, including, specifically, the MPY578fc
cells described in *Pausch et al. (1998)*.

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Yeast strains containing wild type and IC3Δ cholecystokinin CCKB receptor were grown overnight in 2 ml synthetic complete liquid medium containing glucose (2%) and lacking uracil (SCD-ura) medium. In this agar-based plate bioassay, molten (50°C) SCD-ura-his agar medium (35 ml, adjusted to pH 6.8 by addition of

5   concentrated KOH or NH₄OH prior to autoclaving) containing 0.5 mM AT (3-aminotriazole) was inoculated with the overnight culture (2×10^4 cells/ml) and poured into square (9 x 9 cm) petri plates. Solutions of CCK agonists in DMSO (1 mM, 10 μl) were applied to the surface of the solidified agar (Upper left: CCK8S; upper right, CCK8US; lower left, CCK5; lower right, CCK4). Compounds applied to the surface

10   of the plate diffused radially from the site of application and bound to CCKB receptors expressed on the surface of cells embedded in the agar, resulting in induction of *FUS1-HIS3* expression. The responding cells formed a dense growth zone readily detectable over the limited growth of cells observed in response to basal *FUS1-HIS3* expression. Plates were incubated at 30°C for 3 days (Figure 3). Figure

15   3A demonstrates the robust growth response of yeast cells containing the IC3Δ cholecystokinin CCKB receptor, while Figure 3B shows only limited growth by yeast cells containing the wild type CCKB receptor, indicating that the deletion of portion of the third intracellular loop of the CCKB receptor improves its function in yeast.

20   **Example 4.   Functional Expression of a Mutated Rat Somatostatin Receptor (SSTR) in Yeast**

The third intracellular loop participates in many GPCR functions, including G protein coupling, desensitization and interaction with diverse modifying proteins. Somatostatin receptors are encoded in five subtypes, labeled SSTR1-5. Several amino

25   acids are found in the third intracellular loop of the SSTR3 subtype, but not in the equivalent region of SSTR2 subtype. Since SSTR2 functions efficiently in yeast, deletion of those amino acids from IC3 may impart this functional efficiency upon SSTR3. Thus, 8 amino acids, Gln-Trp-Val-Gln-Ala-Pro-Ala-Cys (SEQ ID NO: 15), were deleted from the third intracellular loop of the rSSTR3 cDNA, enabling more

30   efficient receptor signaling in yeast.

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Rat SSTR3 sequences were amplified by PCR using oligonucleotides containing 5' BglII and 3' XhoI sites. The resulting PCR product of approximately 1.3 kb was digested with BglII and XhoI, purified and inserted between the BamHI and XhoI sites in p426GPD to generate the plasmid p426GPD-rSSTR3. Recombinant

5   plasmids were confirmed by restriction endonuclease digestion and DNA sequencing.

Standard PCR reactions were used to amplify the rSSTR3 cDNA to yield two PCR fragments that have 36 bp overlap as follows. PCR insert A of approximate size 750 bp was generated using the 5' Bgl oligonucleotide (AAAAAGATCT AAAATGGCCA CTGTTACCTA T) (SEQ ID NO: 16) and the 3' oligonucleotide

10   CTCAGAGCGG CGTCGCCGCT GACACGAGGG CGCCCG (SEQ ID NO: 17). PCR insert B of approximate size 530 bp was generated using the 5' oligonucleotide GCGCCCTCGT GTCAGCGGCG ACGCCGCTCT GAG (SEQ ID NO: 18) and the 3' XhoI oligonucleotide (AAAACTCGAG TTACAGATGG CTCAGTGTGC T) (SEQ ID NO: 19). PCR fragments A and B were gel purified,

15   annealed and amplified by PCR using the flanking 5' BglII and 3' XhoI oligonucleotides to yield the ~1.3 kb rSSTR3ΔIC3 PCR product. Following purification and digestion with BglII-XhoI, the rSST3ΔIC3 insert was ligated into BamHI-XhoI sites of p426GPD to generate the plasmid p426GPD-rSSTR3ΔIC3. Restriction mapping and DNA sequencing confirmed correct reading frame and

20   sequence.

Yeast cells of the type useful for expression of GPCRs, described in United States Patent 5,691,188, were transformed with p426GPD-rSSTR3 and p426GPD-rSSTR3ΔIC3, using standard procedures. The cells (i.e., the LY296 cells, described in *Price et al. (1995)*) were assayed using the agar-based bioassay format described in

25   Example 3. Samples (10 μl) of Somatostatin (S-14, 1mM) were applied to the surface of the selective agar medium containing the yeast cells expressing the SSTR3. The plates were incubated for 3 days at 30°C. Yeast cells transformed with p426GPD-rSSTR3 along with pLP82 (containing a Gpal/Gαi2 chimeric G-protein expression plasmid) showed a weak growth response to S-14 (Figure 4A), whereas a much

30   stronger response was observed when p426GPD-rSSTR3ΔIC3 was assayed under

- 16 -

similar conditions (Figure 4B). These results indicate that deletion of a portion of the IC3 improves the function of the SSTR3 in yeast.

Example 5.   An IC3 deleted Human Alpha2A Adrenergic Receptor

5

As shown in Examples 1-4, deletion of portion of the IC3 of mammalian GPCRs is correlated with improved functional expression in mammalian and yeast cells with retention of full ability to couple to the heterotrimeric G protein. The mutated MARs, CCKBR, and SSTR3 retain all external loops. Transmembrane
10   domains and internal domains other than the IC3 are unchanged. The IC3, found between 5th and 6th membrane spanning helices, was the only domain modified. The bulk of this domain was deleted leaving only 22 amino acids proximal to both the 5th and 6th transmembrane helices. Thus, IC3 of the GPCRs containing the IC3 deletion (IC3Δ) is 44 amino acids in length. The improvement in functional expression may
15   be due to elimination of domains known to interact with cellular desensitization mechanisms, allowing more functional MAR to be retained at the cell surface.

In order to test the possibility that other IC3Δ mutations would also improve functional expression of other GPCRs in yeast, DNA sequences encoding an IC3Δ human alpha2A adrenergic receptor were amplified by PCR and cloned into proximity
20   to the glycerol-phosphate dehydrogenase promoter in yeast expression plasmid, p426GPD, by standard methods. The IC3Δ human alpha2A adrenergic receptor was prepared by fusing two overlapping fragments. Fragment 1 contained amino terminal coding sequences including 39 amino acids proximal to the 5th TMD, amplified by PCR using oligonucleotides
25   (GGCCAGGATCCAAAAATGGGCTCCCTGCAGCCGGACGC) (SEQ ID NO: 20) and (CGGGCCCCGCGGGCGCTCGGGGCCCAGACCGTTGGGC) (SEQ ID NO: 21). Fragment 2 contained carboxy terminal coding sequences including 41 amino acids proximal to the 6th TMD, amplified by PCR using oligonucleotides (CGGGCGACAGCCTGCCGCGGC) (SEQ ID NO: 22) and
30   (AGCGGTCGACTCACACGATCCGCTTCCTGTCCCC) (SEQ ID NO: 23). The two fragments were fused by amplification by PCR using oligos at 5' and 3' ends of the full length alpha2A adrenergic receptor. Using a conventional lithium acetate

- 17 -

transformation procedure, the resulting plasmids were introduced into yeast cells useful for performing assays of GPCR agonist-stimulated growth, such as those described in United States Patent 5,691,188, including, specifically, the MPY578fc cells described in *Pausch et al. (1998)*.

5   Yeast cells containing the wild type IC3Δ human alpha2A adrenergic receptor were assayed in liquid culture using the alpha adrenergic receptor full agonist UK14304 (RBI) and partial agonist clonidine. The cells were cultured overnight in 2 ml SC-glucose-ura medium. The cells were diluted 500 fold in SC-glucose-ura-his, pH 6.8 medium. Samples of the cell suspension (180 μl) were dispensed to wells of

10   sterile 96 well microtiter dishes containing 20 μl of serially-diluted samples of the adrenergic receptor agonist, UK14304 (10^{-3} - 10^{-10} M). The plates were incubated at 30°C for 18 hours with agitation (600 rpm). Growth was monitored by recording increases in OD620 using a microplate reader. Assays were conducted in duplicate and growth rate measurements obtained during the logarithmic phase of yeast cell

15   growth. Optical density measurements were analyzed using GraphPad Prism and are presented as the mean ± SEM and were plotted vs. agonist concentration.

The yeast cells containing the wild type IC3Δ human alpha 2A adrenergic receptor produced a dose-dependent growth response, indicating that this IC3 deletion is functional (Figure 5).

20

**Example 6.   Truncation of the Rat Neurotensin NT1 Receptor
                 Causes an Increase in Agonist Sensitivity**

In examples 1-4, modification of the third intracellular loop leads to

25   improvement in functional expression of a variety of heterologous GPCRs expressed in yeast. Agonist induced desensitization of the GPCRs is also mediated in part by GPCR internal domains other than the third intracellular loop, such as the intracellular carboxy-terminal tail.

Elimination of the carboxy terminal domains from GPCRs has been shown to

30   improve functional expression in yeast and mammalian cells. Truncation of the carboxy terminal tail of the G protein-coupled alpha mating pheromone receptor expressed in α-mating type yeast cells causes supersensitivity to the presence of

- 18 -

mating pheromone (*Reneke et al. (1988); Konopka et al (1988)*). Consistent with these observations, a mutated rat neurotensin NT1 receptor (rNTR1) lacking its carboxy terminal tail is resistant to agonist-induced internalization when expressed in mammalian cells (*Hermans et al. (1996)*).

5   To test whether carboxy-terminal truncation improves the functional response of a heterologous GPCR expressed in yeast, the rat NTR1 was modified by deleting the 52 amino acids that constitute the carboxy terminal tail, leaving a shortened receptor 372 amino acids in length. The coding sequences of the wild type and truncated neurotensin NT1 receptor (rNTR1 C-term Δ), were amplified by PCR using

10   a 5' oligonucleotide primer that specified a common amino-terminal coding sequence (AGTCAGATCTAAGCTT AAAA ATG CAC CTC AAC AGC TCC) (SEQ ID NO: 24) and separate oligos that define the wild type (AGTC AGATCT CTA GTA CAG GGTCTCCC) (SEQ ID NO: 25) and truncated carboxy termini (AGAG AGATCT TTAGCGCCACCCAGGACAAAGGC) (SEQ ID NO: 26). These fragments were

15   cloned into proximity of the PGK promoter in the yeast expression vector pPGK by standard methods (*Y-S. Kang et al. (1990)*). Using a conventional lithium acetate transformation procedure, the resulting plasmids were introduced into yeast cells of the kind described in United States Patent 5,691,188 that are useful for performing assays of GPCR agonist-stimulated growth, including, specifically, the MPY578fc

20   cells described in *Pausch et al. (1998)*.

Yeast cells containing the NTR1s were assayed in liquid culture using the NT receptor agonist acetyl neurotensin 8-13 (AcNt8-13). The cells were cultured overnight in 2 ml SC-glucose-ura medium. The cells were diluted 500 fold in SC-glucose-ura-his, pH 6.8 medium containing 2 mM 3-aminotriazole to decrease basal

25   growth rate. Samples of the cell suspension (180 µl) were dispensed to wells of sterile 96 well microtiter dishes containing 20 µl of serially-diluted samples (10^{-3}-10^{-10} M) of AcNT8-13. The plates were incubated at 30°C for 18 hours with agitation (600 rpm). Growth was monitored by recording increases in OD_{620} using a microplate reader. Growth rate measurements were obtained during the logarithmic phase of

30   yeast cell growth. Optical density measurements were analyzed using GraphPad Prism and are presented as the mean ± SEM and were plotted against agonist

- 19 -

concentration. As shown in Figure 6, the yeast cells containing the NTR1s produced

an agonist-dependent growth response demonstrating that both the wild type and

carboxy terminally truncated NTR1s were functional. The growth response of the

rNTR1 C-term Δ containing cells was dose-dependent giving an EC50 for AcNT8-13

5   equal to 520 nM. This value is five fold lower than observed for cells expressing the

wild type NTR1 (2.1 μM). The carboxy terminal deletion has produced a rNTR1 that

responds to a lower concentration of NTR agonist improving the sensitivity of the

yeast bioassay.

Thus, deletion of a portion of the carboxy terminal intracellular domain of the

10   rat NTR1 has produced a functional GPCR with increased agonist sensitivity when

expressed in yeast, suggesting that modification of this internal domain is a

generalizable method for improving the function of heterologous GPCRs expressed in

yeast.

15   **Example 7.   Functional Expression of a Mutated *C. elegans* Serotonin Receptor
       in Yeast**

Agonists of the G protein-coupled *C. elegans* serotonin receptor (Ce 5HTR)

may possess substantial nematacidal activity. These observations suggest that

20   development of a yeast-based HTS for agonists active at Ce 5HTRs may be useful in

identifying lead compounds that might be developed into nematacides with a novel

mode of action. Preliminary experiments indicated that the wild type Ce 5HTR was

non-functional in yeast. Thus, an effort to develop a method for improving the

function of the Ce 5HTR in yeast was mounted, via replacement of the Ce 5HTR IC3

25   with the functional IC3 from the M3 MAR IC3Δ.

In order to construct the IC3 replacement, PCR fragments encoding three

domains were prepared by standard means. Fragment 1 contains the amino terminal-

coding portion of the Ce 5HTR to the intracellular interface of the 5th TMD,

amplified by PCR using oligonucleotides

30   (AAAAGATCTAAAATGATCGACGAGACGCTTC) (SEQ ID NO: 27) and

(CCGCTTGGTGATCTGACTTCTGGTTTCTGTCCCAGAGGCCTGTAGGCCAG

CCAGCTCTTTGGTACGCTTCTCAGTTTCCTTATAGATCTTCCAGTACACGC

- 20 -

AAATTATTGC) (SEQ ID NO: 28). The second fragment of the Ce 5HTR contains
carboxy-terminal coding sequences starting proximal to the intracellular interface of
the 6th TMD, amplified by PCR using oligonucleotides
(AAAACTCGAGTCAATAATCGTGAATAAGGCA) (SEQ ID NO: 29) and
5 (GGCCTACAGGCCTCTGGGACAGAAACCAGAAGTCAGATCACCAAGCGGA
AGAGGATGTCGCTCATCAAGGAGAAGAAGGCCGCCAGAACGCTAGCAAT
TATTACAGGTAC) (SEQ ID NO: 30). The M3 MAR IC3Δ domain was amplified
by PCR as described in Example 1.

These fragments were isolated, mixed and amplified by PCR with 5'
10 (AAAAGATCTAAAATGATCGACGAGACGCTTC) (SEQ ID NO: 27) and 3'
(AAAACTCGAGTCAATAATCGTGAATAAGGCA) (SEQ ID NO: 29)
oligonucleotides. The
resulting fragment was digested with appropriate restriction endonucleases and
assembled in p426GPD to place the mutated Ce 5HTR under control of the GPD
15 promoter. Using a conventional lithium acetate transformation procedure, the
resulting plasmids were introduced into yeast cells useful for performing assays of
GPCR agonist-stimulated growth, such as those described in United States Patent
5,691,188, including, specifically, the MPY578fc cells described in *Pausch et al.
1998.*

20 Yeast cells containing the Ce 5HTR were assayed in liquid culture. The cells
were cultured overnight in 2 ml SC-glucose-ura medium. The cells were diluted 500
fold in SC-glucose-ura-his, pH 6.8 medium containing 2 mM 3-aminotriazole to
decrease basal growth rate. Samples of the cell suspension (200 μl) were dispensed to
wells of sterile 96 well microtiter dishes containing 2.0 μl of serially-diluted samples
25 (10^{-2} - 10^{-9} M) of serotonin (5HT). In similar reactions, the serotonergic antagonists
lisuride and mianserin were added to each well at 10 μM. The plates were incubated at
30°C for 18 hours with agitation (600 rpm). Growth was monitored by recording
increases in OD620 using a microplate reader. Assays were conducted in duplicate
and growth rate measurements were obtained during the logarithmic phase of yeast
30 cell growth. Optical density measurements were analyzed using GraphPad Prism and
are presented as the mean ± SEM, plotted vs. agonist concentration. Ki values were

- 21 -

determined using the equation of Cheng and Pruesoff. *(Y. Cheng et al. (1973))*. As

shown in Figure 7, the yeast cells containing the mutated Ce 5HTR containing the M3

MAR IC3Δ produced an agonist-dependent growth response, demonstrating that the

Ce 5HTR-M3 MAR IC3Δ is functional. As expected, the serotonergic antagonists

5   lisuride and mianserin blocked the growth inducing effect of serotonin, demonstrating

that the Ce 5HTR exhibits the expected pharmacological properties when expressed in

yeast.

Thus, replacing IC3 of the Ce 5HTR with the functional deleted IC3 from the

rat M3 MAR produces a functional chimeric GPCR when expressed in yeast,

10   indicating that this method of replacing internal domains may be a generalizable

method for improving the functional expression of heterologous GPCRs in cell-based

assays, such as yeast assays.

Other embodiments of the invention will be apparent to those skilled in the art

from consideration of the specification and practice of the invention disclosed herein.

15   It is intended that the specification and examples be considered as exemplary only,

with a true scope and spirit of the invention being indicated by the following claims.

The references cited herein are specifically incorporated by reference in their

entirety.

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We claim:

1. An isolated nucleic acid sequence having a sequence encoding a modified G protein-coupled receptor (GPCR), wherein the modification comprises a mutation in an intracellular domain of the G protein-coupled receptor and results in an improved functional response in a cell-based assay, and wherein the modified G protein-coupled receptor is selected from the group consisting of a muscarinic acetylcholine receptor, a cholecystokinin CCKB receptor, a somatostatin receptor, an alpha 2A adrenergic receptor, and a serotonin receptor.

2. The nucleic acid sequence according to claim 1, wherein the modification promotes agonist stimulated growth, and wherein the agonist is a G protein-coupled receptor agonist.

3. The nucleic acid according to claim 2, wherein the modification results in improved coupling between the receptor and a heterotrimeric G protein or failure of the receptor to interact with cell desensitization or sequestration-internalization machinery or proper plasma membrane localization.

4. The nucleic acid according to claim 1, wherein the mutation is a deletion.

5. The nucleic acid according to claim 4, wherein the deletion is a point mutation.

6. The nucleic acid according to claim 4, wherein the deletion is in the third intracellular loop of the G protein-coupled receptor.

7. The nucleic acid according to claim 6, wherein the G protein-coupled receptor is selected from the group consisting of a muscarinic acetylcholine receptor, a cholecystokinin CCKB receptor, and an alpha 2A adrenergic receptor.

8. The nucleic acid sequence according to claim 1, wherein the serotonin receptor is Ce 5HTR.

9. The nucleic acid sequence according to claim 1, wherein the muscarinic acetylcholine receptor is a rat M3 muscarinic acetylcholine receptor or a *D. melanogaster* muscarinic acetylcholine receptor.

10. The nucleic acid sequence according to claim 1, wherein the cholecystokinin CCKB receptor is a rat cholecystokinin CCKB receptor.

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11. The nucleic acid sequence according to claim 1, wherein the somatostatin receptor is a rat somatostatin receptor subtype 3.

12. The nucleic acid sequence according to claim 1, wherein the alpha 2A adrenergic receptor is a human alpha 2A adrenergic receptor.

13. A modified G protein-coupled receptor encoded by the nucleic acid sequence according to claim 1.

14. The G protein-coupled receptor according to claim 13, wherein the deleted third intracellular loop is 44 amino acids in length.

15. A mutant G protein-coupled receptor encoded by the nucleic acid sequence according to claim 10, wherein a sequence Gln-Trp-Val-Gln-Ala-Pro-Ala-Cys (SEQ ID NO:15) is deleted from the third intracellular loop of the mutant G protein-coupled receptor.

16. An isolated nucleic acid having a sequence encoding a chimeric G protein-coupled receptor, wherein the chimeric G protein-coupled receptor comprises a modified intracellular domain of a G protein-coupled receptor that confers an improved functional response to the chimeric G protein-coupled receptor in a cell-based assay.

17. The nucleic acid according to claim 16, wherein the modified intracellular domain is the third intracellular loop.

18. A vector comprising the nucleic acid sequence according to claim 1 or 16.

19. A host cell transformed with a vector according to claim 10.

20. The host cell according to claim 19, further comprising a plasmid comprising an inducible reporter gene.

21. The host cell according to claim 19, wherein the host cell is a eukaryotic cell.

22. The host cell according to claim 21, wherein the eukaryotic cell is a yeast cell.

23. The host cell according to claim 22, further comprising a plasmid comprising an inducible reporter gene.

24. The host cell according to claim 23, wherein the reporter gene is a green fluorescent protein.

25. The host cell according to claim 24, wherein the green fluorescent protein is operably linked to a *FUS2* promoter.

26. A method for screening compounds capable of binding to G protein-coupled receptors comprising:

(a) subjecting the host cell according to claim 19 to a test compound; and

(b) measuring the effect of the test compound on cell growth.

27. A host cell comprising a heterologous G protein-coupled receptor, wherein the G protein-coupled receptor has a modification that results in an improved functional response of the G protein-coupled receptor in a cell-based assay.

28. The host cell according to claim 27, wherein the modification promotes agonist stimulated growth, and wherein the agonist is a G protein-coupled receptor agonist

29. The host cell according to claim 28, wherein the modification results in improved coupling between the heterologous G protein-coupled receptor and a heterotrimeric G protein or failure of the heterologous G protein-coupled receptor to interact with cell desensitization or sequestration-internalization machinery or proper plasma membrane localization.

30. The host cell according to claim 27, wherein the host cell is a eukaryotic cell.

31. The host cell according to claim 30, wherein the heterologous G protein-coupled receptor is modified at an intracellular domain.

32. The host cell according to claim 31, wherein the intracellular domain is the third intracellular domain.

33. The host cell according to claim 27, wherein the heterologous G protein-coupled receptor is modified at the carboxy terminal tail of the G protein-coupled receptor.

34. The host cell according to claim 31 or 33, wherein the host cell is yeast.
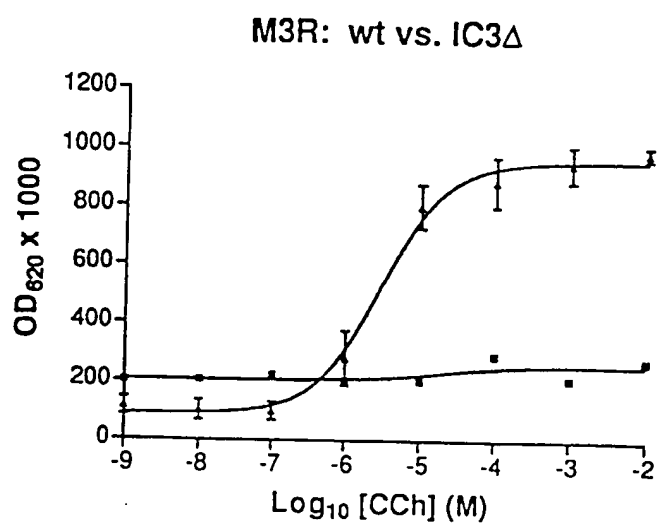
35. The host cell according to claim 34, wherein the modified G protein-coupled receptor is a neurotensin receptor.

36. The host cell according to claim 35, wherein the neurotensin receptor is a rat neurotensin NT1 receptor.

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37. A method for screening compounds capable of binding to G protein-coupled receptors comprising:
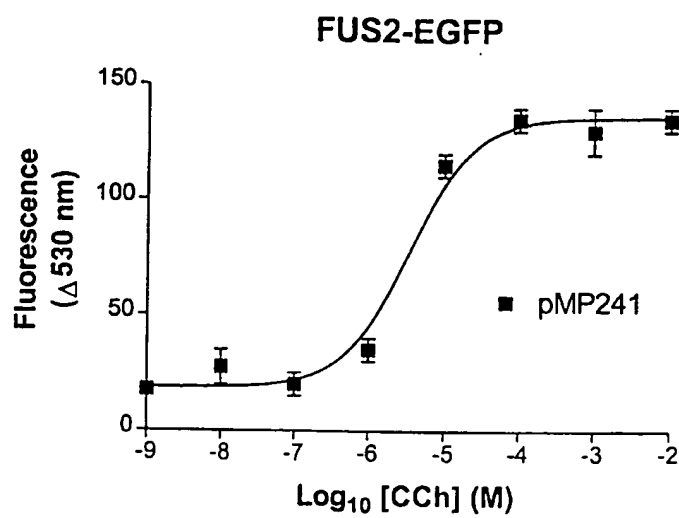
(a) subjecting the host cell according to claim 27 to a test compound; and

(b) measuring the effect of the test compound on yeast cell growth.
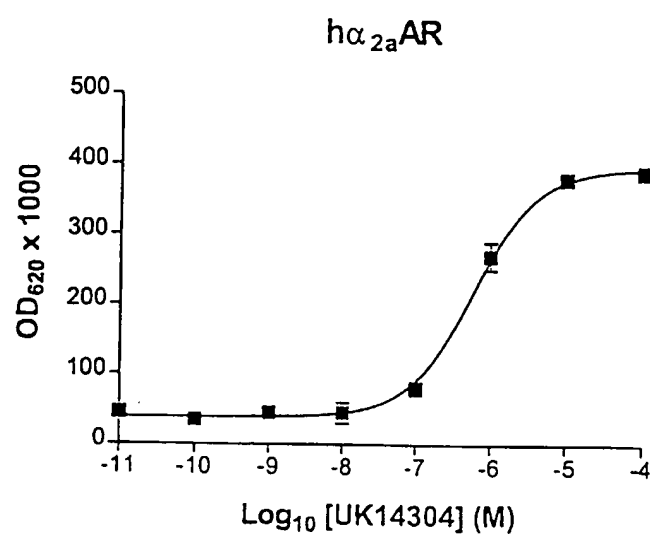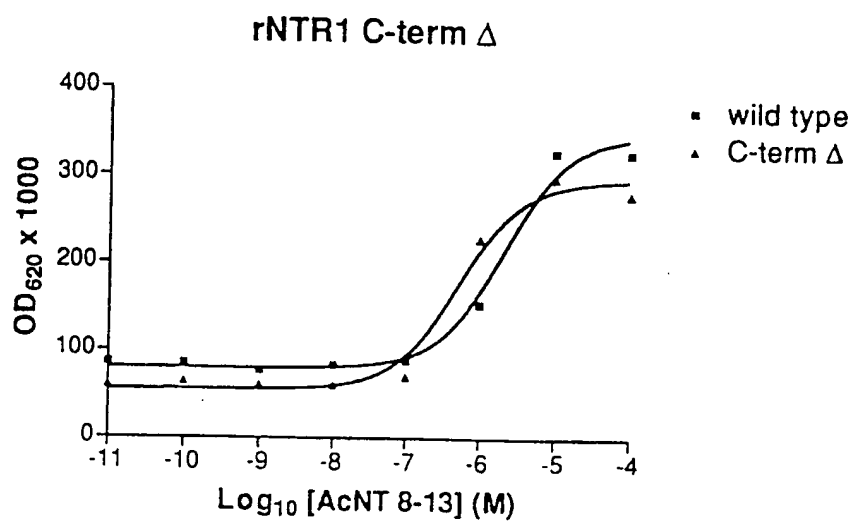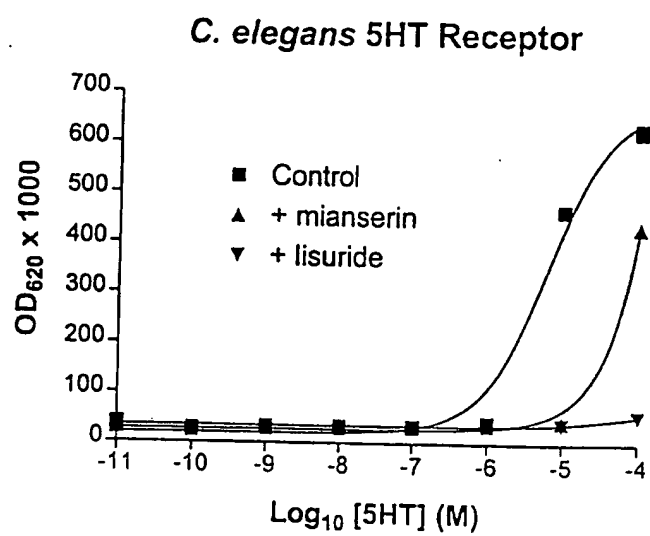
FIG. 1A

FIG. 1B

FIG. 2

FIG. 3

A

B

FIG. 4

FIG. 5

FIG. 6

FIG. 7